

STUDIES OF *ERYTHRINA* ALKALOIDS, PART III.¹
G.C./M.S. INVESTIGATIONS OF ALKALOIDS IN THE
SEEDS OF A FURTHER FOURTEEN SPECIES²

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ABSTRACT

Fourteen species of *Erythrina* were characterized for the total alkaloids content, both in the free form and as glycosides, by gas chromatography-mass spectrometry. Thirteen alkaloids were identified in all, the most common being erysodine, erysovine, and erysopine. A number of unknown or unidentified were also noted.

In two previous papers (Games et al., 1974; Barakat et al., 1977) we described preliminary studies of the occurrence of alkaloids in the seeds of some 26 species of *Erythrina*. In the present work we have continued using gas chromatography-mass spectrometry (g.c./m.s.) to analyze the alkaloid content of 14 further species, and as in the earlier studies (Barakat et al., 1977), we have characterized the total alkaloids occurring both in the "free" form and as their glycosides; the latter were hydrolyzed to enable the "liberated" alkaloids to be analyzed. Hydroxyl groups were derivatized as their trimethylsilyl (TMS) derivatives before g.c./m.s., and the individual alkaloids present in each species were characterized by comparisons of their mass spectra with those of authentic materials isolated from other species, and with data reported in the literature. A number of new alkaloids were also detected in the course of this work, but full characterization will depend on the availability of sufficient seeds for preparative isolation of the alkaloids concerned.

ALKALOIDS IN *ERYTHRINA* SPECIES

The results of the present studies with the seeds of 14 species are shown in Table 1, and for comparison earlier findings by other workers with nine of these species are recorded in Table 2. The previous work depended largely on classical separation methods, i.e., crystallization and column chromatography, the one exception being Rinehart's investigations (Hargreaves et al., 1974) of *Erythrina flabelliformis* seeds, which like our own work involved g.c./m.s. As indicated in the list of sources of seeds used in our studies, several different samples of seeds collected at different times (some were more than 30 years old), or in different

¹ Our previous two papers (Games et al., 1974; Barakat et al., 1977) are regarded as parts I and II.

² We are very grateful to B. A. Krukoff for supplying all the seeds used in this work, and for his keen interest in the results. We are also pleased to record our gratitude for a grant towards this work from the F. B. and B. A. Krukoff Memorial Fund, and we thank the S.R.C. for assistance in the purchase of the mass spectrometer used in this work. We thank Mr. M. Rossiter for assistance in running the mass spectra.

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TABLE 1. Occurrence of alkaloids in 14 species of *Erythrina* seeds examined in Cardiff.

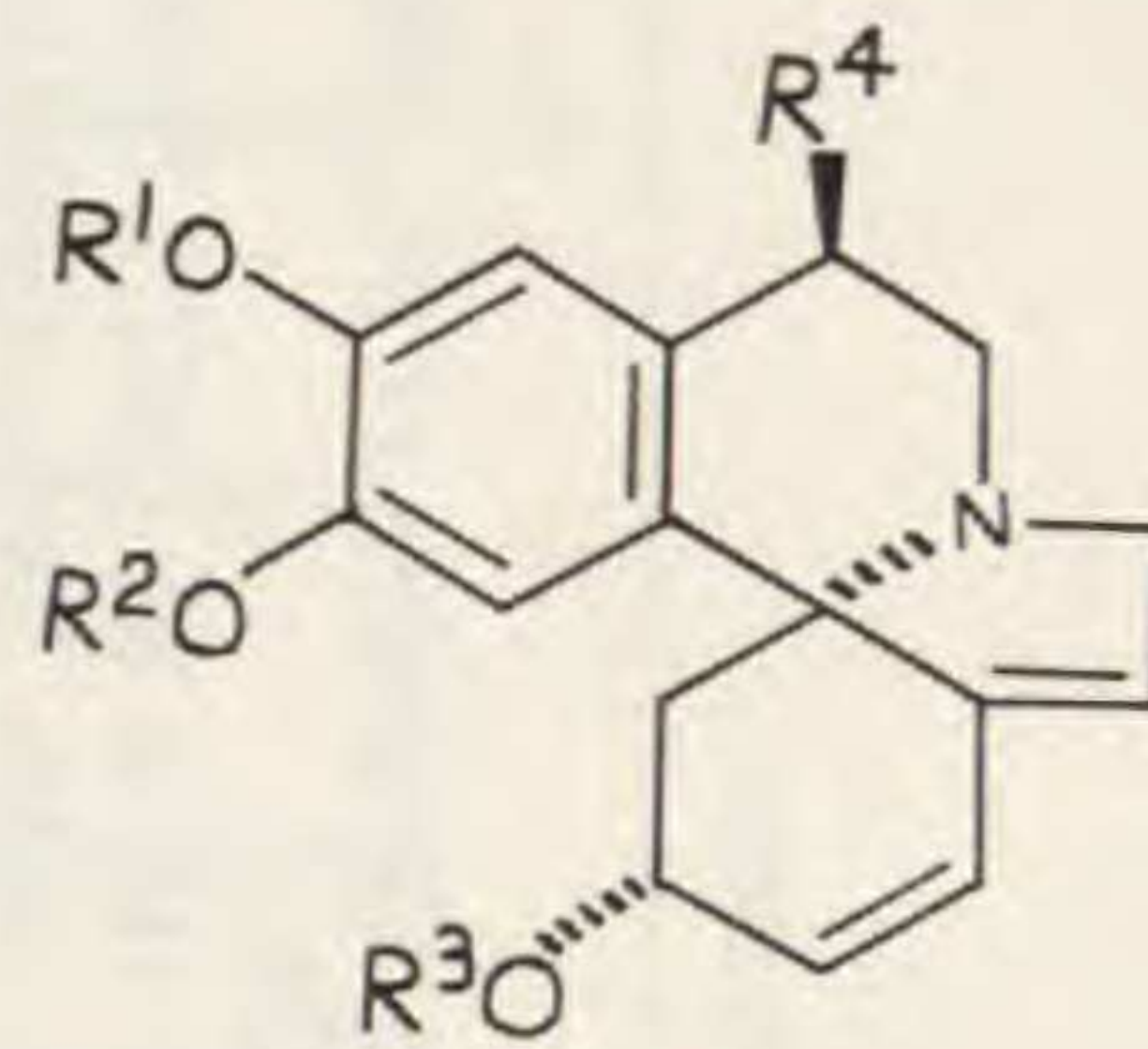
Species of Seeds	No. of Samples	Dienoid						Alkenoid					No. of Unknown/ Unidentified Alkaloids		
		Erysoitrine (1)	Erysodine (3)	Erysovine (4)	Erythravine (5)	Erysovine (7)	Erysonine (8)	Erysoline (9)	Erythratidine (11)	Erysovine (13)	Erysoalvine (14)	α -Erythroidine (17)		β -Erythroidine (18)	Erybidine (20)
2. <i>E. crista-galli</i>	2	+	+	++		++			++					+	3
3. <i>E. falcata</i>	1		+	+		++		+	+						2
4. <i>E. dominguezii</i>	1		+	+		++		++	+	+					1
6. <i>E. verna</i>	4		++	++		++		+	+	+	+				2
8. <i>E. suberosa</i>	1		+++	+		++									3
14. <i>E. breviflora</i>	2		+	+	+	++									1
22. <i>E. herbacea</i>	4		++	++		++			++	+				3	
24. <i>E. flabelliformis</i>	6		+	+		++		+							2
51. <i>E. americana</i>	1			+		++					+++	+++			1
58. <i>E. amazonica</i>	1		+	+		++									3
62. <i>S. pallida</i>	1		+	+		++									3
87. <i>E. addisoniae</i>	1		+	+		++		+					+		1
90. <i>E. saclexii</i>	1		+	+		++		+							1
101. <i>E. velutina</i>	3		+	+		++		+							2

^a Erybidine only found in sample (a).^b Erythratidine and erysovine were only found in sample (b).

TABLE 2. Occurrence of alkaloids in *Erythrina* species determined in other laboratories.

Species and Parts	Dienoid						Alkenoid										References
	Eryso-trine (1)	Ery-thra-line (2)	Ery-so-dine (3)	Ery-so-vine (4)	Ery-thri-nine (6)	Ery-so-pine (7)	Ery-thra-mine (10)	Ery-thra-tidine (11)	Ery-thra-tine (12)	Ery-thra-tinone (15)	Christa-midine (19)	α -Ery-throidine (17)	β -Ery-throidine (18)	Ery-bidine (20)	Hypa-phorine (22)	N-noro-rientaline (21)	
2. <i>E. crista-galli</i>																	
seeds	+	+	+	+		+	+	+									
leaves	+	+	+		+									+			
heart wood, bark, roots	+	+			+			+	+	+	+		+		+	+	
3. <i>E. falcata</i> seeds			+	+		+											
4. <i>E. dominguezii</i> seeds			+	+		+											
8. <i>E. suberosa</i> seeds, leaves	+		+			+								+			
22. <i>E. herbacea</i> seeds			+			+								+			
24. <i>E. flabelliformis</i> seeds			+	+		+											
seeds	+		+	+		+											
51. <i>E. americana</i> seeds			+	+													
62. <i>E. pallida</i> seeds			+	+		+						+	+				
101. <i>E. velutina</i> seeds		+	+	+													

TABLE 3. Structures of dienoid alkaloids.

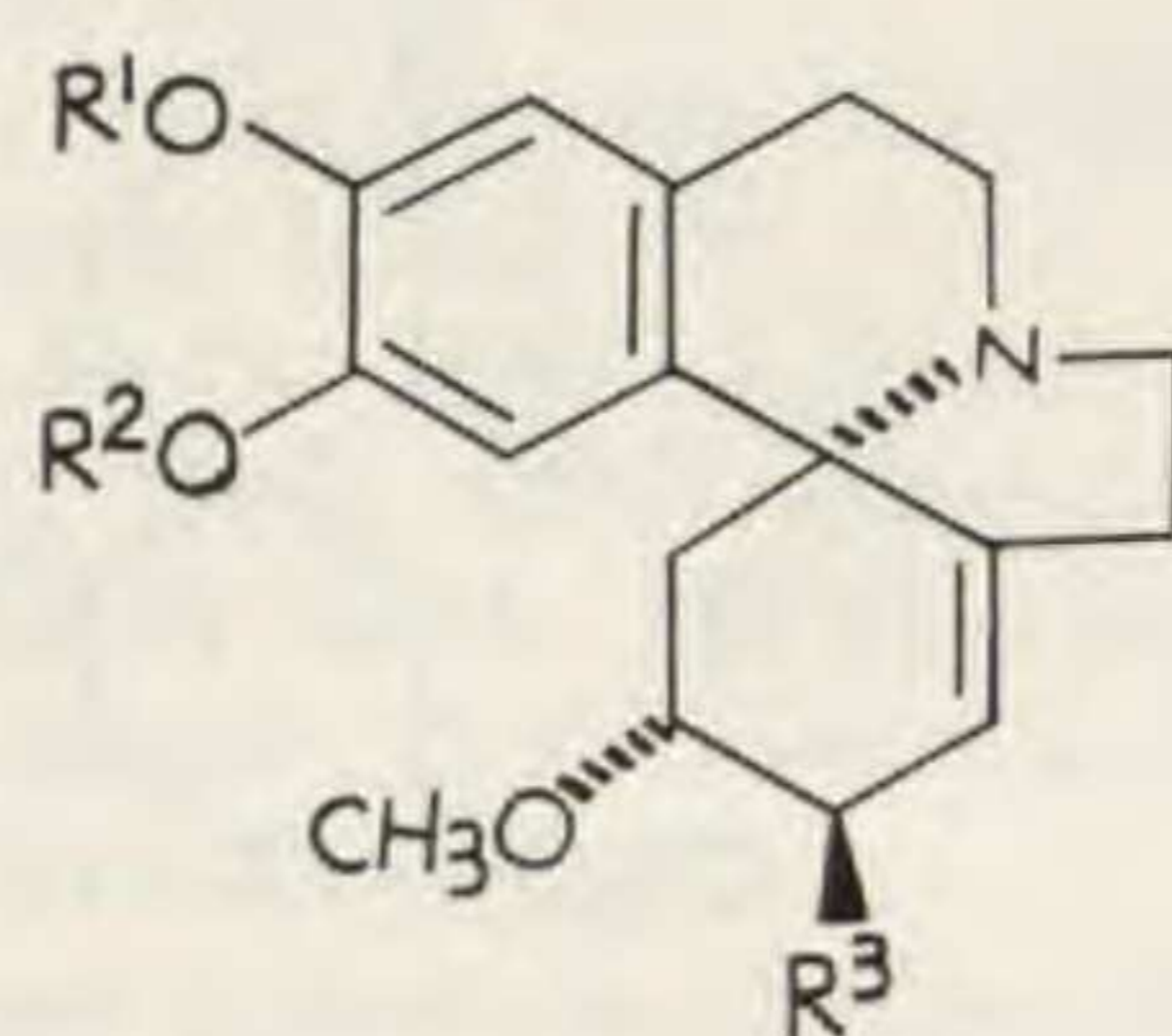


	R ¹	R ²	R ³	R ⁴
(1) Erysotrine	CH ₃	CH ₃	CH ₃	H
(2) Erythraline		-CH ₃ -	CH ₃	H
(3) Erysodine	H	CH ₃	CH ₃	H
(4) Erysovine	CH ₃	H	CH ₃	H
(5) Erythravine	CH ₃	CH ₃	H	H
(6) Erythrinine		-CH ₃ -	CH ₃	OH
(7) Erysopine	H	H	CH ₃	H
(8) Erysonine	H	CH ₃	H	H
(9) Erysoline	CH ₃	H	H	H

places, were available with some of the species. Small variations between the alkaloid content of different samples of the same seeds are also noted in Table 1, and there are also minor differences between our findings and those of earlier workers shown in Table 2. The structures of the various alkaloids are shown in Tables 3, 4 and 5.

The most common alkaloids, and also usually present in greatest abundance in all the species investigated, were erysodine (3) (numbers in parenthesis refer to the compounds listed in Tables 1-5), erysovine (4) and erysopine (7). However, in accord with earlier work (Folkers & Koniuszy, 1940b; Folkers & Major, 1937), the most abundant alkaloids (>90%) in *Erythrina americana* were α - and β -erythroidines (17) and (18), although small amounts of erysodine, erysovine and erysopine were also detected. Our results also showed that erysonine (8), erysoline (9), erythratidine (11) and erysotine (13) occurred in about half the species studied. Erysosalvine (14) was only found in two species, *E. dominiguezii* and *E. verna*, whilst erythravine (5) and erybidine (20) were each only found in one species (*E. breviflora* and *E. crista-galli* respectively). Although several alkaloids were found in *E. addisoniae*, these included only one of the erythroidine isomers (18), whereas in other species containing erythroidines both isomers are normally present (cf. Games et al., 1974; Barakat et al., 1977; Hargreaves et al., 1974), although the proportions may vary. As in the earlier studies, the isomeric alkaloids erysonine (8) and erysoline (9) could not be distinguished from each other by mass spectrometry, but their TMS derivatives had slightly different retention times on gas chromatography, that of erysonine (8) being eluted first.

TABLE 4. Structures of alkenoid alkaloids.



	R ¹	R ²	R ³
(10) Erythramine		-CH ₂ -	H
(11) Erythratidine	CH ₃	CH ₃	OH
(12) Erythratine		-CH ₂ -	OH
(13) Erysotine	H	CH ₃	OH
(14) Erysosalvine	CH ₃	H	OH
(15) Erythratinone		-CH ₂ -	=O

A number of unknown alkaloids (as specified in Table 1) were also detected in each species, mostly in rather smaller amounts than the three most abundant alkaloids. Mass spectral evidence, based on the trimethylsilyl derivatives, indicated that some of these unknown alkaloids contained one, or more, hydroxyl groups; in the case of *Erythrina suberosa* preliminary indications of the presence of 11-hydroxy and 11-methoxy alkaloids were also obtained.

It is interesting to note from Table 2 that since our original report indicating that 11-oxygenated alkaloids might be largely confined to Old-World species of *Erythrina*, Japanese workers (Ito et al., 1973) have described the occurrence of erythrinine (6) in *E. crista-galli*. However, their studies were carried out with plants cultivated in Japan, doubtless one of the numerous cultivated forms of this species. We failed to detect erythrinine in wild plants of American origin.

Further work on the isolation and characterization of both the known and unknown alkaloids in each species is in progress (where sufficient seeds are available) using high pressure liquid chromatography.

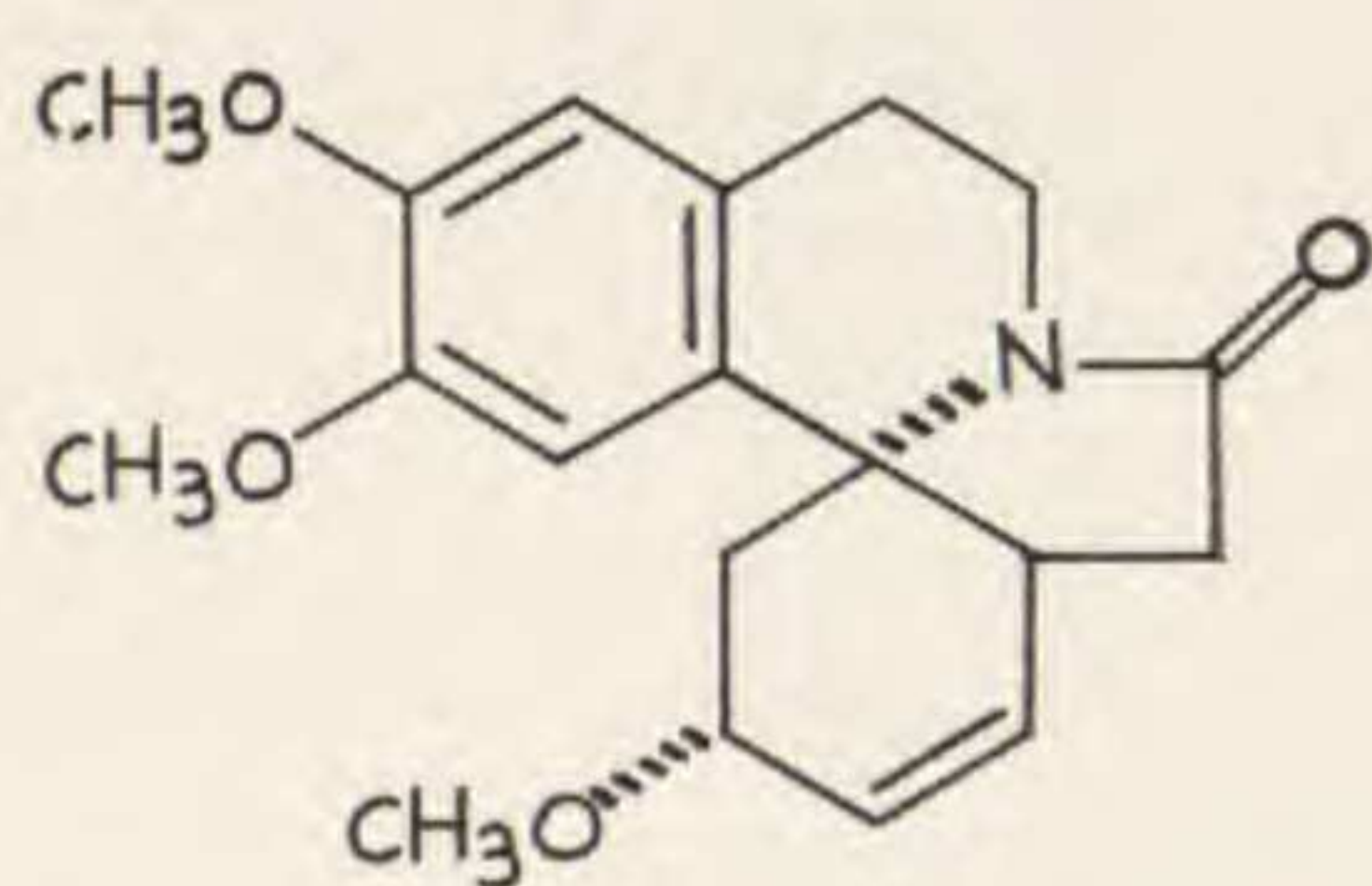
SOURCES OF *ERYTHRINA* SEEDS

All the seeds used in these studies were supplied by B. A. Krukoff and most are backed by herbarium material deposited at the New York Botanical Garden and other herbaria.

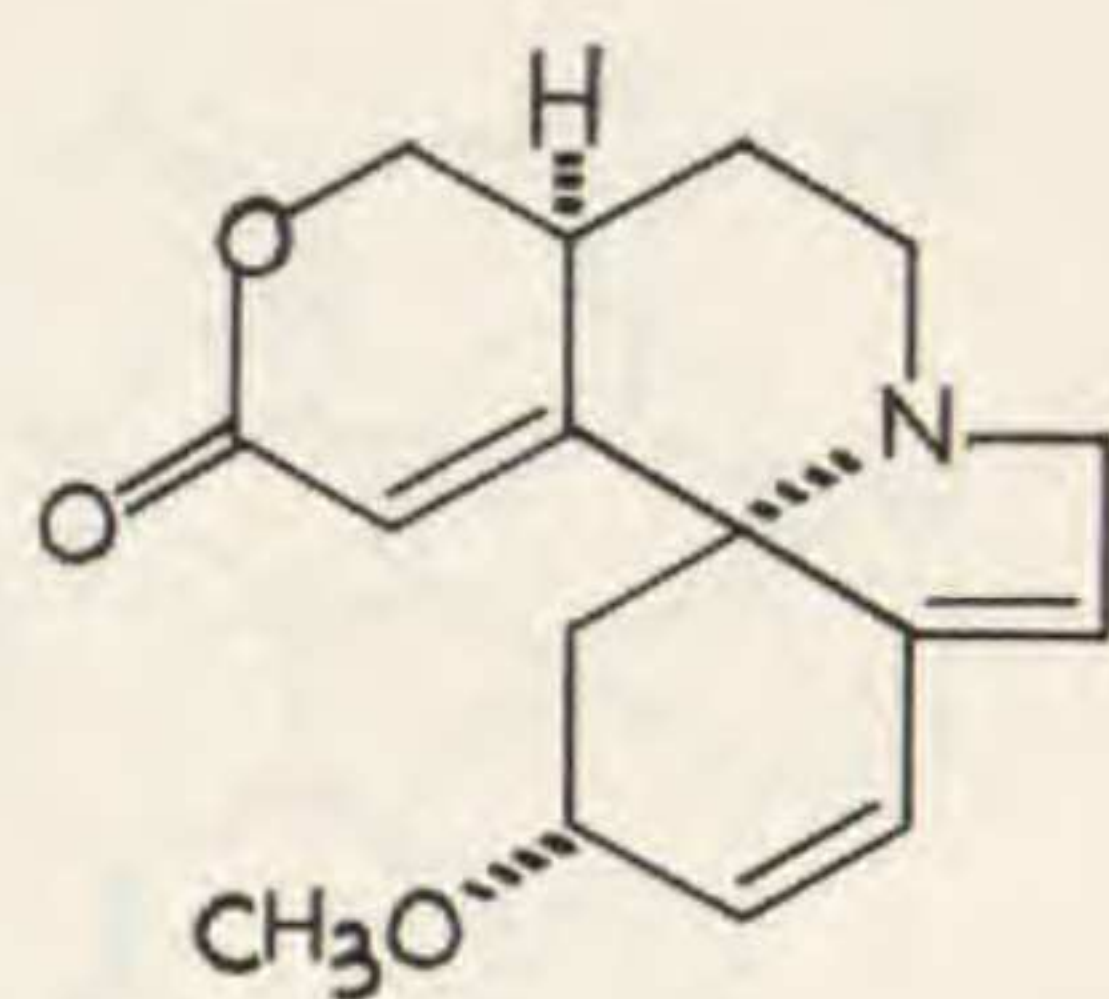
2. *E. crista-galli* L.; (a) Meyer s.n./1974—California.
(b) Krukoff Herb. 9187⁵—Argentina.

⁵ Seed samples marked "Krukoff Herb." are very old, having been collected in the early 1940s in connection with studies of alkaloids in the genus by K. Folkers and others.

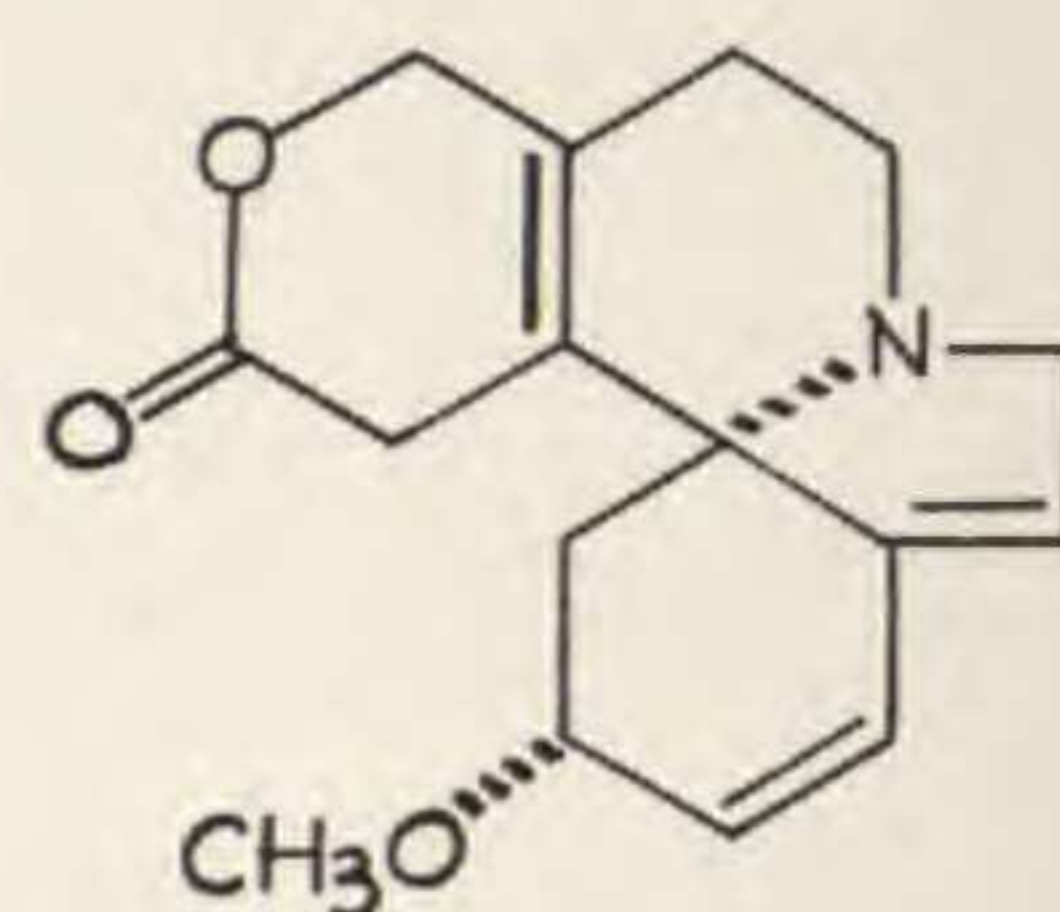
TABLE 5. Structures of other alkaloids.



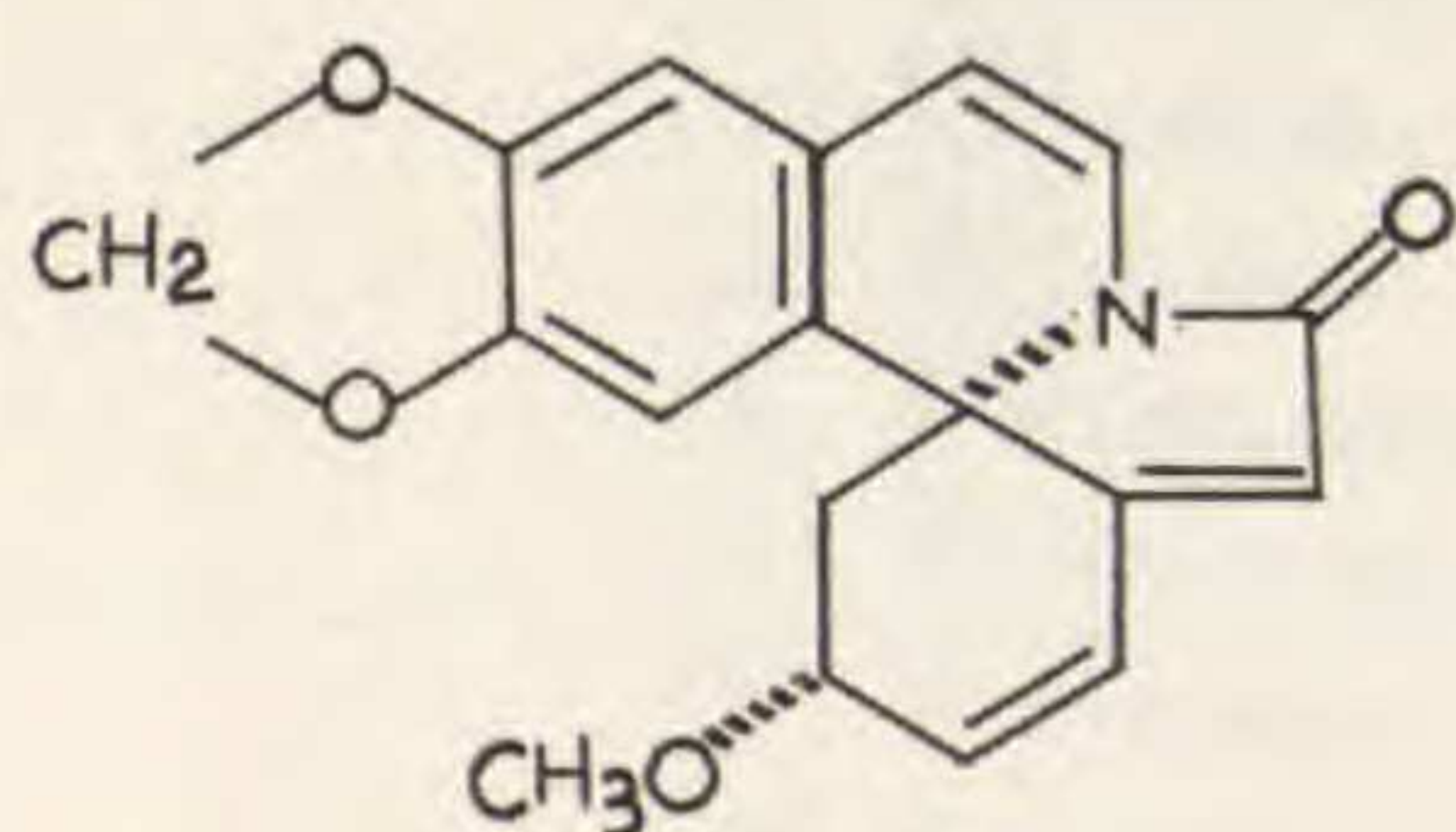
(16)



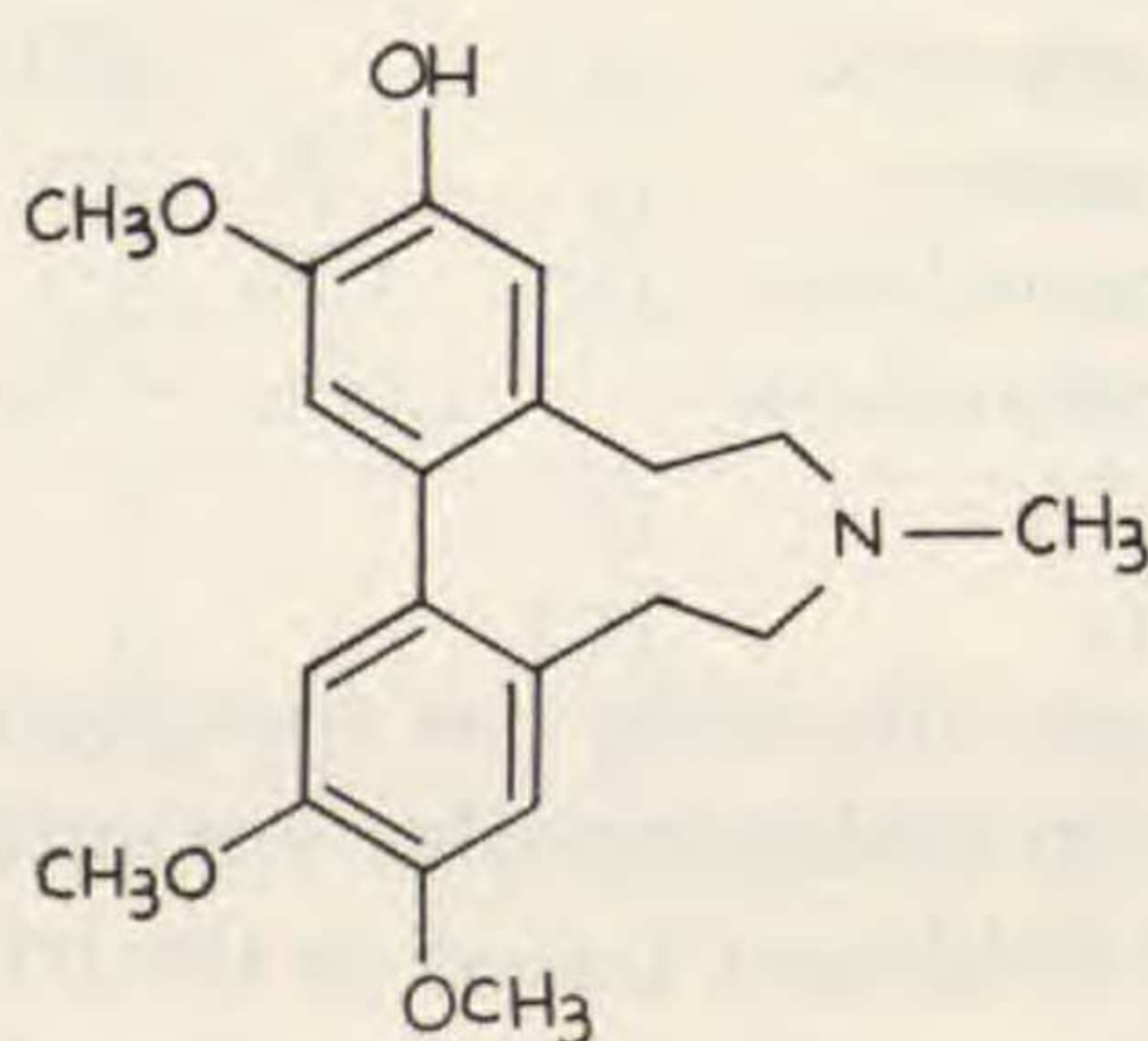
(17)



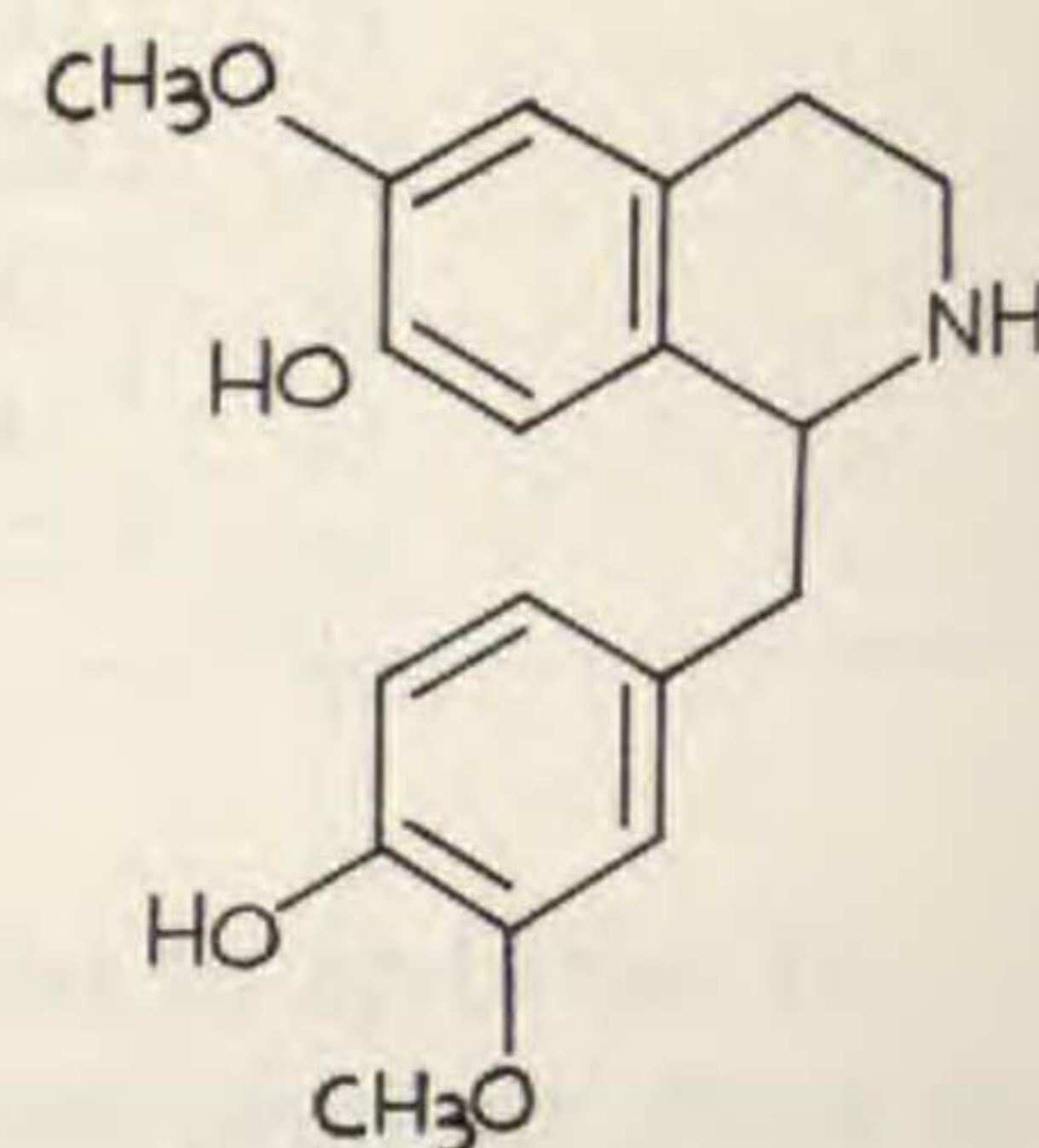
(18)



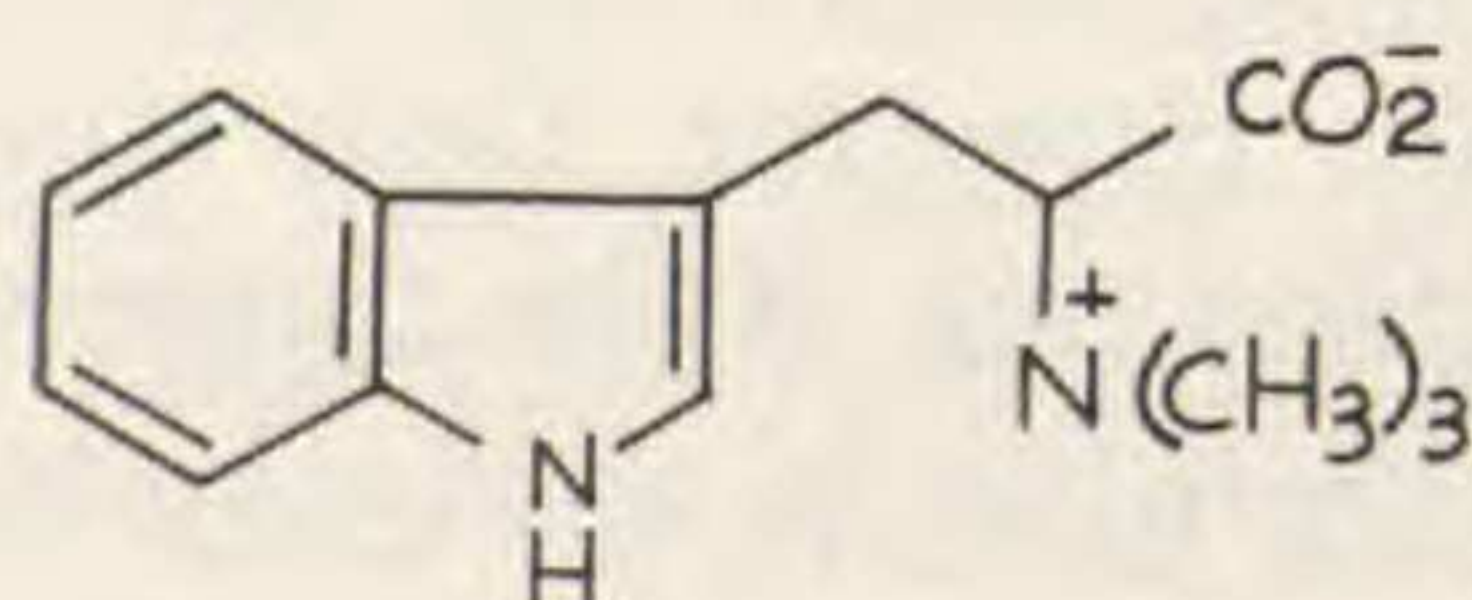
(19)



(20)



(21)



(22)

3. *E. falcata* Benth; Krukoff Herb. 15396—Buenos Aires, Argentina.
 4. *E. dominguezii* Hassler; Krukoff Herb. 15378—Chaco, Argentina.
 6. *E. verna* Velloso; (a) Krukoff Herb. 16347—Argentina.
 (b) Krukoff Herb. 17732—Brazil.
 (c) Krukoff Herb. 16352—Brazil.
 (d) Krukoff Herb. 16665—Brazil.
 8. *E. suberosa* Roxburgh; Krukoff Herb. 9864—India.
 14. *E. breviflora* A. DC.; (a) Krukoff Herb. 15346—Mexico.

- (b) Krukoff Herb. 15379—Mexico.
- 22a. *E. herbacea* L. subsp. *herbacea*; (a) Krukoff Herb. 16627—Louisiana, U.S.A.
 (b) Krukoff Herb. 15578—Texas, U.S.A.
 (c) Krukoff Herb. 18883—Florida, U.S.A.
 (d) Clifton s.n./1978—Florida, U.S.A.
24. *E. flagelliformis* Kearney; (a) Sally's seeder s.n./1975—Arizona, U.S.A. (5 samples).
 (b) Lyle Angus McGill—Arizona, U.S.A.
52. *E. americana* Miller; Krukoff Herb. 16881—Veracruz, Mexico.
59. *E. amazonica* Krukoff; Krukoff Herb. 9515—Maranhão, Brazil.
63. *E. pallida* Britton & Rose; Krukoff Herb. 15183—Trinidad.
88. *E. addisoniae* Hutchinson & Dalziel; A.A. Enti s.n./1978—Kumasi, Ghana.
91. *E. sacleuxii* Hua; Faden 74/294—Kenya.
102. *E. velutina* Willd.; (a) Dardano Lima s.n./1974—Brazil.
 (b) Dardano de Andrade s.n./1974—Brazil.
- 102a. *E. velutina* forma *aurantica* (Krukoff) Ridley; Lima s.n./1974—Brazil.

EXPERIMENTAL METHODS

The alkaloids were isolated from the seeds by similar methods to those used previously (Games et al., 1974; Barakat et al., 1977; Deulofeu et al., 1947), and the mass spectra were determined with a Varian CH 5D mass spectrometer coupled with a Varian 1740 gas chromatograph via a two stage Watson-Biemann separator. The temperature of the ion source was 220°C and the accelerating and ionising potentials were 3 Kv and 70 eV respectively. Spectra were recorded electrically using a Varian 620i data system, and after subtraction of background peaks were normalized and plotted on a Statos 21 fast printer. Field ionization and desorption mass spectrometry were used for preliminary studies of the crude alkaloid mixtures before chromatography.

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